PROJECT NUMBER: 1904
PROJECT TITLE: Tobacco Biochemistry
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PERIOD COVERED: June, 1989

I. LOW NICOTINE STUDY

A. **Objective:** To investigate the biochemistry of the nicotine biosynthetic pathway at the putrescine N-methyltransferase (PMT) step and specifically to isolate PMT from tobacco root extracts.

B. **Status:** Tobacco plants from Group 18 were harvested and the roots and leaves (top, mid-stalk, and bottom) from 3, 5, 6, and 7 days after topping were stored at -80°C. Three roots (about 1 kg) from each of the 4 time periods were processed through the 40-65% ammonium sulfate stage and the extracts were assayed for PMT activity to find the optimal time for root harvest, which coincides with maximal PMT activity. These results will be compared with the data obtained from a similar study with Group 17 plants (1).

Four 100 ml aliquots of M-8-phenyl-Sepharose extract were applied to an SAH column for purification of PMT by affinity chromatography. The PMT active fractions were eluted with 1 mM SAM and stored at -80°C. for further use (2). Fifty ml of SAH-Sepharose was prepared for purifying additional PMT extracts (2,3).

Cytochrome c was chosen as an agent to minimize PMT-activity losses due to denaturation and/or nonspecific adsorptions in dilute PMT preparations. Amicon ultrafiltration and Centricon cells (not dry Sephadex-G-25) were found to be satisfactory methods for the concentration of PMT. A DEAE-5PW (TosoHaas) column with HPLC was observed to give an 100% yield of PMT activity. One mg of PMT preparation (M8-Φ-SAH-4) was concentrated and applied to a series of HPLC columns (anion exchange, hydroxyapatite, hydrophobic interaction, and gel permeation). The fractions containing PMT activity were pooled and concentrated after each column and before application to the next column. SDS-PAGE examination of fractions from the last column (gel permeation) indicated low protein levels of a nonhomogeneous preparation (4).

ELFE (preparative electrophoresis) investigations included the determination of the proper running conditions and loading efficiencies for different size gels. Preliminary experiments indicate that individual standard proteins may separate into specific fractions after elution from the gel. Studies are under way to check the feasibility of renaturing PMT after exposure to SDS (5).

Work has commenced on the isolation of total RNA from tobacco root and leaf tissue. Root tissue from tobacco plants grown hydroponically have been frozen and shipped to Stratagene for a cDNA library construction. A memo has been written on the use of differential hybridization for the isolation of root specific genes (6).

C. **Plans**: Process root and leaf material from Group 18 plants. Purify M-8-Φ extracts through SAH columns for use in other studies. Explore the four HPLC columns in series for the isolation of purified PMT. Confirm the sensitivity of the Protein Gold assay by the addition of standard protein to test solutions. Continue studies with ELFE and protein renaturation. Isolate, purify, and characterize RNA from tobacco roots.

D. **References**:

1. Lyle, J. Notebook No. 8397.
2. Mooz, E. D. Notebook No. 8803.
3. Yu, T. Notebook No. 8806.
4. Nakatani, H. Y. Notebook No. 8384.
5. Davies, S. M. Notebook No. 8761.
6. Malik, V. S. Notebook No. 8274.